WHAT WE CLAIM IS:

1. Method of processing digital images comprising one or more objects to be quantified, the method comprising the following main stages:

- normalization of the digital images;

- quantization of the images to one bit, further comprising at least one of the following stages:

- calculating, from the images quantized to one bit, the perimeter, area and/or fractal dimension of the one or more objects to be quantified;

- reconstructing, from the images quantized to one bit, a 3D-image of the one or more objects to be quantified, and/or

- calculating, from the normalized images, the fractal dimension of the overall image.

2. Method according to claim 1, the method comprising a stage of image's normalization (NORM) which comprises the following steps:

1) dividing the image into quadrants;

2) calculating the mean value of intensity of the pixels belonging to each quadrant;

3) calculating the mean value of intensity for all the quadrants as a mean of the calculated means of step 2);

4) setting for each quadrant the mean value of

intensity calculated according to step 3) by performing one of adding or subtracting a same intensity value to each pixel inside a quadrant in order to maintain the original $\Delta_{intensity}$ among the pixels inside a same quadrant;

5) reiterating steps 1) to 4) up to a preset quadrant side length.

3. Method according to claim 2, wherein the preset quadrant side length is approximately half length of the minor side of the one or more objects to be quantified.

4. Method according to claim 1, wherein the digital image has been acquired by a confocal microscopy.

5. Method according to claim 4, wherein the confocal microscopy is a Laser Scanning Confocal Microscopy (LSCM) or a Scanning Ophtalmoscopy.

6. Method according to claim 1, which comprises the following steps:

1a) dividing the image into four quadrants;

2a) calculating the mean value of intensity of the pixels belonging to each quadrant;

3a) calculating the mean value of intensity for the four quadrants as a mean of the four calculated means of step 2a);

4a) setting for each quadrant the mean value of intensity calculated according to step 3a) by performing one of adding or subtracting a same intensity value to each pixel inside a quadrant in order to maintain the original $\Delta_{intensity}$ among the pixels inside a same quadrant;

5a) determining for each quadrant the max and the min values of intensity of the pixels and calculating for each pixel an extended intensity value (EI) which derives from the stretching of the digital values inside the range of the possible digital values;

6a) setting for each pixel the EI_{pixel} calculated according to step 5a);

7a) reiterating steps 1a) to 6a) up to a preset quadrant side length.

7. Method according to claim 6, wherein the step 5a) of calculating the EI value of the pixels is performed by means of the following algorithm:

$$EI_{pixel} = (I_{pixel} - I_{min}) \times N / (I_{max} - I_{min})$$

wherein I_{pixel} is the intensity of each pixel of a given quadrant, I_{min} is the min value of intensity of the pixel inside the quadrant, I_{max} is the max value of intensity of the pixel inside the same quadrant and N is an integer more than 1 and up to 255, preferably 255.

8. Method according to claim 1, wherein the

normalization stage comprises:

1b) dividing the image into quadrants;

2b) determining for each quadrant the max and the min values of intensity of the pixels and calculating for each pixel an extended intensity value (EI) which derives from the stretching of the digital values inside the range of the possible digital values;

3b) storing the EI_{pixel} value for each pixel of each quadrant in a data structure;

4b) reiterating steps 1b) to 3b) up to a preset quadrant side length in order to obtain for each pixel a set of intensity values in the data structure;

5b) calculating for each pixel the mean of the intensity values of the set stored in the data structure and setting the calculated mean value to the respective pixel.

9. Method according to claim 8, wherein the step 5a) of calculating the EI value of the pixels is performed by means of the following algorithm:

$$EI_{pixel} = (I_{pixel} - I_{min}) \times N / (I_{max} - I_{min})$$

wherein I_{pixel} is the intensity of each pixel of a given quadrant, I_{min} is the min value of intensity of the pixel inside the quadrant, I_{max} is the max value of intensity of the pixel inside the same quadrant and N is an integer more than 1 and up to 255, preferably 255.

10. Method according to claim 1, further comprising a stage of image elaboration (IMA-EL stage) to quantize the image to "1 bit".

11. Method according to claim 10, wherein the IMA-EL stage comprises the following steps:

1c) considering a parameter for each pixel;

2c) comparing said pixel's parameter with a preset threshold value or threshold range for said parameter;

3c) selecting a cluster of active pixels and a cluster of inactive pixels on the base of said comparison,

wherein said pixel's parameter is preferably brightness intensity (black and white images) or digital colour value.

12. Method according to claim 1, further comprising a stage of image quantification which comprising at least one of the following steps:

- calculating the area A of the object under examination by counting the number of pixels belonging to the cluster of active pixels selected according to the previous IMA-EL stage;

- calculating the perimeter P of the object under examination by i) selecting the object contour's pixels, and ii) applying to such selected pixels a perimeter calculation's algorithm, wherein to each active pixel

belonging to the object is given a "perimeter value", which is a function of the position of the active pixels adjacent to the pixel under examination, the sum of said "perimeter values" being the overall perimeter P of the object.

13. Method according to claim 1, further comprising a stage of object's sorting (SORT) for identifying objects made up from 4-connected pixels, which includes the following steps:

1d) scanning of the image quantized to "1 bit" along a predefined direction on a x, y axis system;

2d) selecting a first active pixel along said direction of scanning, said active pixel being identified by a first set of x, y values, said first active pixel belonging to a first object's image;

3d) performing on said first selected active pixel a search routine in the positions next to said selected pixel on the direction's line;

4d) iterating step 3d) until an inactive pixel is found;

5d) assigning to each active pixel selected according to such steps 3d) and 4d) a set of x, y values, saving them in the storing means of the processing system (7) and switching said pixels from active to inactive in the object's image;

6d) evaluating for each pixel selected according to steps 3d), 4d) and 5d) the two next pixels in the direction orthogonal to the scanning direction and selecting the active pixels;

7d) performing, for each of said active pixels selected according to step 6d), the routine of steps 3d) to 5d);

8d) iterating steps 6d) and 7d) until all of the connected pixels belonging to the same object have been saved;

9d) repeating steps 1d) and 2d) until a first active pixel of a further object's image is found;

10d) repeating steps 3d) to 9d) until the whole image has been scanned.

14. Method according to claim 13, wherein said predefined direction in step 1d) is from left to right starting from top to bottom.

15. Method according to claim 13, wherein the stage of object's sorting according to steps 1d) to 10d) is performed for also identifying objects made up from 8-connected pixels, in said stage the step 6d) being modified as follows:

6d) evaluating for each pixel selected according to steps 3d), 4d) and 5d) the two next pixels in the direction orthogonal to the scanning direction and the

two pixels adjacent to each of these latter pixels on the parallel line adjacent to the direction's line and selecting the active pixels.

16. Method according to claim 13, further comprising at least one of the following steps:

1e) calculating the area of each object identified according to the SORT stage by counting the number of pixels belonging to said object's image and multiplying it for the area of each pixel; and/or

2e) counting the number of objects and calculating its density; and/or

3e) calculating the mean area of the objects by adding the areas calculated according to step 1e) of all the objects sorted and dividing the total area by the number of objects obtained according to step 2e).

17. Method according to claim 1, further comprising a step of calculating a parameter (w) indicating the degree of "rugosity" of the selected object, the (w) parameter being preferably calculated by means of the following algorithm:

$$w = \frac{Pf}{2\sqrt{Af.\pi}} - R$$

wherein Pf is the perimeter, Af is the area of the object and R is the "roundness coefficient" of the object; wherein R is on its turn calculated with the following algorithm

$$R = \frac{Pe}{2\sqrt{Ae.\pi}}$$

wherein Pe is the perimeter of the ellipse in which the measured object is inscribed and Ae its area.

18. Method according to claim 1, further comprising a stage of dimensional calculation (DIM-CLC) for calculating the fractal dimensions of perimeter and area of the observed objects, wherein said fractal dimension of the perimeter (D_P) and said fractal dimension of the area (D_A) are determined according to the following steps:

a) dividing the image of the object into a plurality of grids of boxes having a side length ε, in which ε varies from a first value substantially corresponding to the side of the box in which said object is inscribed and a predefined value which is a fraction of said first value,

b) calculating a value of a logarithmic function of N(ε), in which N(ε) is the number of boxes necessary to completely cover the perimeter (P) or the area (A), respectively, of the object and of a logarithmic function of 1/ε for each ε value of step a), thus obtaining a first set of values for said logarithmic function of N(ε) and a second set of values for said logarithmic function of 1/ε,

c) calculating the fractal dimensions (D_P) or

(D_A) as the slope of the straight line interpolating said first set of values for said logarithmic function of N(ε) for the perimeter (P) or the area (A), respectively, versus said second set of values of step b).

19. Method according to claim 1, further comprising a stage of surface quantification (S-QUANT) performed on the image normalized according to the NORM stage, the stage comprising the following steps:

1f) dividing the image in a x, y bidimensional mesh with n x n boxes of side *l*;

2f) dividing the 0-256 grey scale into n subregions having each a 256/n value;

3f) calculating for each box of the x, y bidimensioanl mesh the min and max value of the pixels contained therein and of the pixels that contour the box;

4f) calculating how many subregions of 256/n value are included between the min and max values of the pixels of each box;

5f) calculating the number N(l) of tridimensional boxes of side l that intercepts the image's surface as a sum of the subregions of all the boxes calculated according to step 4f);

6f) reiterating steps 1f) to 5f) with a side length

l' less than l;

7f) by repeating step 6f), generating a first set of values of a logarithmic function of 1/l and a second set of values of a logarithmic function of N(l);

8f) calculating the fractal dimension of the image's surface as the slope of the straight line interpolating said first set of values versus said second set of values of step 7f).

20. Method according to claim 1, further comprising a stage of 3D-reconstruction (3D-R) performed on the image subjected to the IMA-EL stage, the 3D-R stage comprising the following steps:

1g) overlapping each image with the subsequent image along the z axis;

2g) minimizing the difference of brightness and/or colour intensity between overlapping pixels by shifting along the x axis and/or the y axis an image with respect to each other;

3g) repeating steps 1g) and 2g) for each pair of adjacent images.

21. Method according to claim 20, further comprising a stage of object counting (O-COUNT), which comprises the following steps:

1h) scanning of the 3D-image quantized to "1 bit" along a predefined direction on a x, y axis system;

2h) selecting a first active pixel along said direction of scanning, said active pixel being identified by a first set of x, y values, said first active pixel belonging to a first object's image;

3h) performing on said first selected active pixel a search routine in the positions next to said selected pixel on the direction's line;

4h) iterating step 3h) until an inactive pixel is found;

5h) assigning to each active pixel selected according to such steps 3h) and 4h) a set of x, y values, saving them in the storing means of the processing system 7 (all of such pixels will have the same y value and x values in progressive order) and switching said pixels from active to inactive in the object's image;

6h) evaluating for each pixel selected according to steps 3h), 4h) and 5h) the two next pixels in the coplanar direction orthogonal to the scanning direction and the two next pixels along the z axis, in the directions +z and -z, and selecting the active pixels;

7h) performing, for each of said active pixels selected according to step 6h), the routine of steps 3h) to 5h);

8h) iterating steps 6h) and 7h) until all of the

connected pixels belonging to the same object have been saved;

9h) repeating steps 1h) and 2h) until a first active pixel of a further object's image is found;

10h) repeating steps 3h) to 9h) until the whole image has been scanned;

11h) counting of the number of the objects sorted according to steps 1h) to 10h).

22. Method according to claim 21, wherein the predefined direction in step 1h) is from left to right starting from top to bottom.

23. Method according to claim 21, for sorting also 8-connected pixel objects, wherein step 6h) of the procedure depicted in claim 21 is modified as follows:

6h) evaluating for each pixel selected according to steps 3h), 4h) and 5h) the two next pixels in the coplanar direction orthogonal to the scanning direction and the two next pixels along the z axis, in the directions +z and -z, and the two pixels adjacent to each of these pixels on the parallel line adjacent to the direction's line and selecting the active pixels.

24. Method according to claim 1, further comprising a stage of volume calculation (V-CLC) which comprises the following steps:

1i) calculating the area of each object in a first

2D-image corresponding to a first object's section;

2i) multiplying the area calculated according to step 1i) for the distance between the first section's image and the subsequent section's image, taken in the z direction of scanning, wherein an image of the same object is contained;

3i) reiterating steps 1i) and 2i) for each section's image in the order.

25. Method according to claim 24, wherein the overall volume of the objects in the examined tissue is determined as the sum of the single volumes.

26. Method according to claim 24, wherein the volume is calculated as:

$$v = 1/3d(A + a + \sqrt{A.a})$$

wherein d is the distance between the two sections, A is the area of the first object's section and a is the area of the second object's section.

27. A system (1) for acquiring and processing an image including a confocal scanning microscope (2), electronic image acquisition means (6) operatively connected to said microscope (2), a processing system (7) operatively connected with said confocal scaning microscope (2) and said image acquisition means (6), said processing system (7) comprising a processing unit (CPU), storing means which include a RAM working memory

and a hard disk, said processing system (7) running a program (PRG) to perform a method according to claim 1.

28. A software program (PRG) to perform the method according to claim 1.

29. A computer readable support comprising a program (PRG) to perform the method according to claim 1.